CMFRI Booklet Series No. 16/2019

HANDBOOK ON MARINE ORNAMENTAL FISH SEED PRODUCTION

Mandapam Regional Centre of
ICAR - CMFRI
Mandapam Camp
Tamil Nadu – 623 520

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ICAR- Central Marine Fisheries Research Institute

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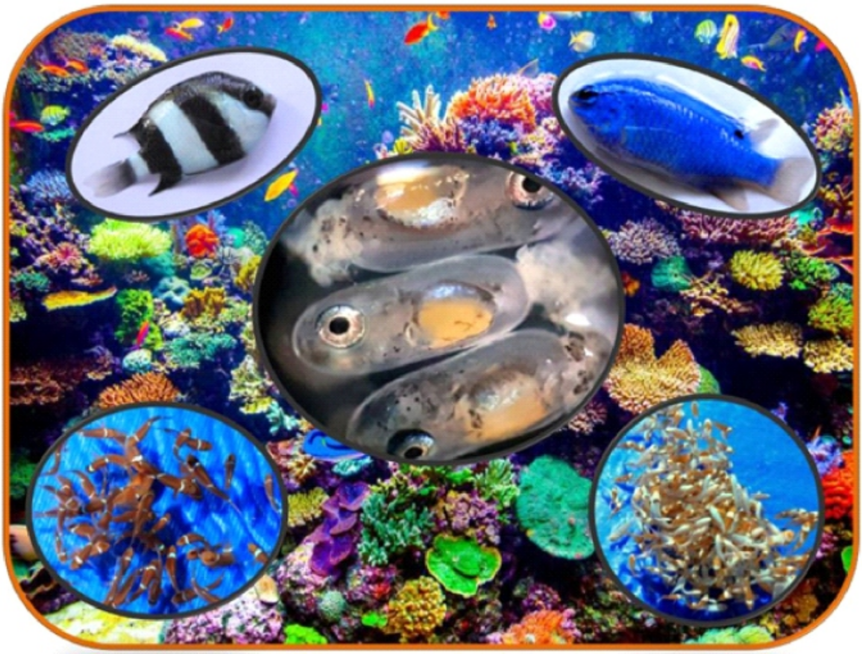

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FOREWORD

The trade in marine ornamentals have been expanding globally owing to the increased demand from the hobbyists. Recent studies (during 1997- 2012) estimated that more than 2500 species are traded comprising 1800 fish and over 700 invertebrate species. The trade is composed of a diverse group of organisms. More than twenty million fish are traded annually, with Pomacentrids (damselfish and clownfish) representing nearly half by volume. The rest is constituted by corals (hard and soft), sea fans, mollusks (gastropods, bivalves, and cephalopods), echinoderms (starfish, urchins, and sea cucumbers), actinarians (sea anemones), crustaceans (shrimp, crabs, and lobsters), polychaetes (feather dusters and Christmas tree worms) and poriferans (sponges). The major exporting countries are the Philippines, Indonesia, Solomon Islands, Sri Lanka, Australia, Fiji, the Maldives, and Palau. The USA is the major importer followed by the United Kingdom, the Netherlands, France, Germany, Taiwan, Japan, China, and Italy.

The marine ornamental fish sector all over the world is dominated by fishes/shell fishes/invertebrates caught from the wild and the share of hatchery produced fishes still continues to be at a very minimal level. The ICAR CMFRI has developed the seed production technologies for more than a dozen varieties of marine ornamental fishes such as clown fishes and damsel fishes. Promoting hatchery production of marine ornamental fishes would be the best option to ensure sustainable development of the sector and imparting training on seed production of marine ornamental fishes will help the coastal fisherfolk to adopt the technology easily. In this context, this handbook on marine ornamental fish seed production would be beneficial for the entrepreneurs and hatchery technicians involved in the marine ornamental fish trade. I congratulate all the persons who had contributed for preparation of this handbook.

Dr. A. Gopalakrishnan
Director, ICAR-CMFRI

PREFACE

Aquarium keeping is a popular hobby and in that the marine ornamentals constitute a major segment in terms of value. However, the share of hatchery produced fishes in the marine ornamental fish trade is very less and wild collection continues to dominate the trade all over the world. Therefore, it is imperative to shift gradually from the wild collection to captive breeding and seed production of marine ornamental fishes. This will ensure sustainability of the marine ornamental trade in the long run. The ICAR CMFRI has developed the technology for breeding and seed production of around fifteen varieties of marine ornamental fishes such as clown fishes and damsel fishes and recently Mandapam Regional Centre of ICAR CMFRI has achieved the mass production of designer clown fishes such as Picasso and Platinum.As a means to help the adoption of the technologies among the costal fisherwomen SHGs of the Gulf of Mannar region, training programmes have been conducted at the centre in collaboration with the GOMBRT (Gulf of Mannar Biosphere Reserve Trust) and more than 150 fisherfolk were trained on marine ornamental fish breeding and seed production. The adoption of these technologies have started in a small way and many fisherwomen SHGs (Self Help Groups) and individuals in the maritime states have already ventured into small scale production of marine ornamental fishes.

In this context, a handbook on marine ornamental fish seed production would be highly useful as a ready reference guide for the trainees, trainers and academicians. I congratulate all those who have put in their efforts to bring out this book.

Dr. R. Jayakumar
Scientist-in charge
Mandapam RC of ICAR CMFRI

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Handbook on Marine ornamental fish seed production

Introduction

Aquarium keeping is a very popular hobby, the benefits of which have been recognized globally. It is a multi- billion dollar industry and it is estimated that globally around 1.5 -2 million people keep aquaria. The trade of marine ornamental organisms and plants has been expanding globally owing to the increased awareness and demand from the hobbyists/consumers. Over 46,000,000 organisms representing 2500 species are traded annually with a value exceeding US$ 300,000,000. Philippines and Indonesia supply the majority of livestock, with most specimens being consumed by the USA, Europe, and Japan (Calado *et al.,* 2017). The marine ornamental fish sector all over the world is dominated by fishes/shell fishes/invertebrates caught from the wild and the share of hatchery produced fishes still continues to be at a very minimal level.

Even on a global basis, the commercial level hatchery production technologies have been evolved only for a limited number of species. The ultimate answer for long term sustainable trade of marine ornamentals can be achieved only through the development of captive breeding and culture technologies. It is well accepted as an environmentally sound way to increase the supply of marine ornamentals by reducing the pressure on wild population and producing juvenile and market sized fish of wide variety of fish year round. Moreover, hatchery produced fish are hardier and fair better in captivity and survive longer than wild caught ones.

The number of captive bred marine aquarium fish, comes to over 250 species (Sweet, 2014). However, for most of these species, it will be quite some time before they can be supplied to the aquarium trade at reasonable price through commercial production. However, to date, successful commercial rearing has been scientifically reported for only a few species. Around 5% of marine aquarium fish traded are commercially produced from hatchery. The main families bred for aquarium purposes are Pomacentridae, Pseudochromidae, Gobiidae, Apogonidae, Pomacanthidae and Syngnathidae (Dominguez and Botella, 2014; Calado *et. al*.2017). The concepts of breeding techniques and larval rearing of freshwater ornamental fishes are mostly not applicable in the case of marine ornamental fishes.

BREEDING AND SEED PRODUCTION

➢ The marine ornamental fishes that are most commonly bred in captivity include clown fishes and damsel fishes. The absence of sexual dimorphism, the complex patterns of sex change in certain groups and the problems of larval rearing can be considered as the major reasons for the slow progress in the culture of marine ornamental fishes. The hatchery protocols for seed production of marine ornamental fishes are more or less similar and the various activities in the seed production can be briefly grouped as follows:

- ➢ Brood stock development
- ➢ Spawning
- ➢ Larval rearing
- ➢ Live feed culture

SEED PRODUCTION OF CLOWN FISHES

Clown fishes are distributed throughout the Indo-West Pacific Region. Clown fishes continue to be the most demanded marine tropical fish and technologies available at present on marine ornamental fish breeding are mainly centered around these fishes. They are distinguished and taxonomically separated from damselfish by their dependence on anemones for protection. They are further distinguished from damsels by their large capsule shaped eggs and large larvae at hatch.

Species of clownfish for which hatchery technologies are developed and standardized in India

Sl.No.	Species name	Common name
1.	*Amphiprion sebae*	Sebae clownfish
2.	*A.clarkii*	Clark's anemone fish / yellowtail clownfish
3.	*A.percula*	Orange clownfish
4.	*A.ocellaris*	Ocellaris clownfish/ false percula clownfish
5.	*A.frenatus*	Tomato clownfish
6.	*A.perideraion*	Pink skunk clownfish
7.	*A.nigripes*	Maldive anemone fish/ black finned anemonefish
8.	*A.ephippeum*	Red saddle anemone fish/ Fire clown
9.	*A.akallopsisos*	Skunk clownfis
10.	*Premnas biaculeatus*	Spine cheeked anemone fish/ maroon clownfish

The Mandapam Regional Centre ICAR CMFRI has also standardized the breeding and larval rearing techniques for designer clown fishes (such as Picasso, Platinum, snowflake, Tear drop etc), which are having high market demand and premium price among clown fishes.

BROOD STOCK DEVELOPMENT

The broodstock development and maintenance is the most important thing. The brood stock of the fishes has to be maintained at optimum water quality conditions (Table 1) free from pathogenic organisms in order to condition them for breeding. This is possible by providing a Recirculatory Aquaculture System (RAS) in the hatchery. The Mandapam Regional Centre of ICAR CMFRI has devised a low cost technique for setting up of mini RAS for maintaining the broodstock of marine ornamental fishes. The basic components include the following:

- A waste water collection sump
- Biofilter
- UV filter
- Chiller for thermal regulation
- Protein skimmer
- Air Blower
- Photo period regulation in fish tanks

The clown fishes are monogamous in nature and pair formation is a difficult part of the captive breeding technique. The clown fishes are born as males and according to social conditions prevailing in the clownfish colony they reverse the sex. There will be only one functional pair (male and female) in a colony. All the other members of the colony remain as sub-adults. In a colony, one pair of fish will grow ahead of other fish – the larger of the two will become the female and the other one will be the male. Hence in a clownfish colony, the larger fish will be the female and the next large fish will be the male. Compatible pairs have to be identified by trial and error methods and once the pair formation is complete, they can be introduced into the brood stock holding tank, such that, only one pair is kept in a tank with a host anemone for better results. Age of the fish is the most important factor determining sexual maturity. Sexually matured adult clownfish are usually 9-18 months old. Conditioning is a prerequisite for spawning any fish. Conditioning is a term used to describe the utilization and manipulation of a combination of environmental factors to induce gonadal maturation and spawning. The factors may include light intensity, light duration and possibly wave length, temperature, water current, water quality, nitrogen, phosphate, ammonia, pH, type of food, tank size and shape, aeration and habitat. All fish do not respond to the same environmental cues which trigger spawning. Under natural conditions, wild clownfish spawn most of the year, but usually not more than one spawn per month. Under optimum conditions and proper feeding, they can be induced to spawn at least twice a month.

The quality of broodstock diets greatly influences successful spawning. Hence suitable diets at satiation levels must be fed to the broodstock fish. Boiled and chopped mussel/clam meat and fish roe can be fed *ad libitum* twice a day. Live feeds like *Artemia* nauplii, adult *Artemia* and *Moina micrura* can also be supplemented. If brood stock fish are not properly fed, the results are directly reflected in the number of eggs laid, pigmentation of the eggs, fertilization rate, hatch rate and the quality of hatched larvae. Poor quality eggs develop slowly, hatch late and often result in significant early larval mortalities.

Table-1: Optimum water quality parameters for marine ornamental fish brood stock development

Sl.No	Parameter	Values
1	Temperature	27-28 °C
2	Salinity	30 – 35 ppt
3	pH	7.5 - 8.5
4	Dissolved Oxygen	> 5ppm
5	Ammonia	0 ppm
6	Nitrite	0 ppm
7	Nitrate	< 25 ppm

SPAWNING

The clown fish spawn by attaching the eggs to any substratum available in the tank. Hence, suitable substratum such as tiles or earthen pots can be placed inside the brood stock tanks. The clownfish normally spawn during forenoon. Once spawning commences, females press their body towards the substrate and slowly move in a rowing fashion using their pectoral fins. She moves in a circular path depositing a continuous spiral of eggs from the central outward. The male swims behind the female, releasing sperm over the newly deposited eggs. Spawning occurs during day time and it lasts for about one to one and half hours. Each female lays 300 to 1000 capsule shaped eggs. Generally the egg size ranges between 1.5 to 3mm in length and 0.8 to 1.8mm in width. Each egg is attached to the substratum by a stalk. During the incubation period both the parents carefully look after the eggs by fanning the eggs by their fins and removing the dead and infected eggs by mouth. After spawning the males assume a more dominant role. He intermittently fans the nest with his caudal or pectoral fins. He also cleans the eggs by gently mouthing them without removing them. Dead and fungal infected eggs are routinely removed and eaten. Substrate around the nest is also often cleaned. The male spends an average of 30-60% of its time during the day for tending the nest. Fanning the eggs is frequent on the day after spawning and diminishes considerably about mid way in the incubation period. On the day of hatch, fanning increases again. In captivity most pairs spawn a minimum of 11 months a year, regardless of the species.

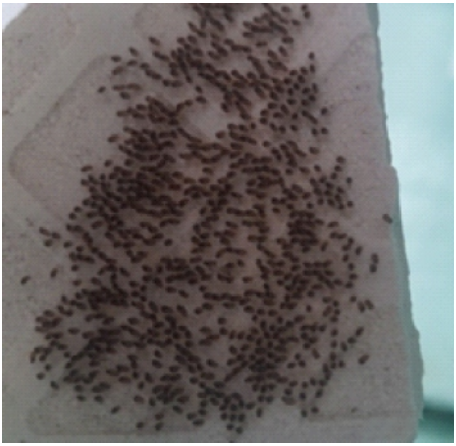
The colour of the eggs will be bright orange initially which will change to black and finally to silvery colour with prominent eyes of the embryo on the 7th day. The eggs hatch on the seventh day shortly after sunset at a water temperature range of 27 – 29 ^{0}C . On the expected day of hatching, 2 hours before sunset the eggs along with the substratum are transferred to hatching tanks. The larvae break their capsules and hatchlings emerge soon after sunset and peak hatching takes place between 1900 – 2000 hrs in darkness.

LARVAL REARING

The newly hatched larvae measures 3-4 mm in length and each has transparent body, large eyes, visible mouth and a small yolk sac. Soon after hatching the larvae are free swimming. The initial nourishment to the developing fish larvae is obtained from the egg yolk. When the yolk reserves have been completely utilized, the larval feeding capabilities are developed and hence at this stage the larval survival is entirely dependent on the availability and quality of food in sufficient quantities. When the yolk reserve depleted, the larvae turn to exogenous feeding for further development is the most critical stage. At this stage, suitable live feed should be available in the larval rearing tanks. Larval rearing has to be carried out in green water (using microalgae such as *Nannochlorposis oculata, N.salina etc*) and feeding with rotifers (*Broachionus plicatilis & B rotundiformis*) initially (upto 8th day after hatching) and then with *Artemia* nauplii from 9th day onwards. A minimum 8-10 numbers of rotifers per ml is required during rotifer feeding period and 2-3 nos nauplii per ml during *Artemia* feeding stage. The larvae metamorphose between 15-20 days. After metamorphosis the larvae can be transferred to grow out tanks with sea anemone. Mild aeration can be provided during larval rearing. The larviculture period from 3-8 dph is critical due to the change in feeding from endogenous to exogenous. After 8 dph there will not be any further mortality if proper feeding and water quality parameters are maintained. The tank bottom should be cleaned daily with at least 25% water exchange. Sufficient green water should be added daily. Weaning with pellet feed can be started after metamorphosis. Start with suitable sized pellets along with the live feeds and slowly wean them to the pellet feed. The ICAR CMFRI has developed a marine ornamental fish feed "Cadalmin ™ **Varna**" which gives very good colour enhancement for the fishes. Boiled and chopped mussel meat can also be used as feed. Two times feeding to satiation level is sufficient to rear them to marketable size.

Brood stock maintenance with mini RAS

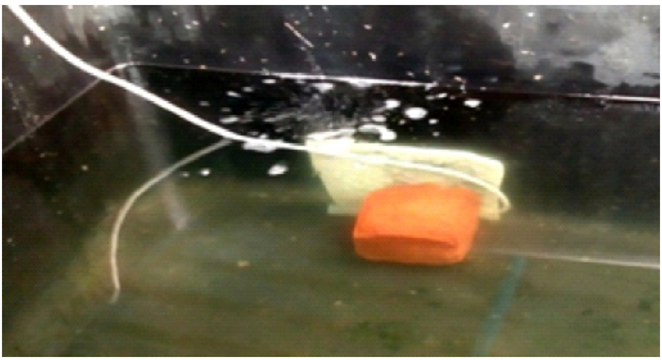
Freshly laid eggs of clown fish



Clown fish eggs on 7th day

Egg with developed embryo of clown fish

Clown fish brooders with freshly laid eggs (arrow) on the tile (Substratum)



Tile (substratum) with eggs kept for hatching

SEED PRODUCTION OF DAMSELFISHES

The damsel fishes which also belong to the Family Pomacentridae are very popular among aquarists due to their small size, bright colours, quick acclimation to captivity and interesting behaviour. The majority of species inhabit the Indo-Pacific region and about 100 species and 18 genera have been recorded from the Indian Ocean. More than 30 species belonging to the genera *Pomacentrus, Neopomacentrus, Chromis, Abudefduf* and *Chrysiptera* are commonly available from Indian coral seas.

Broodstock development and larval rearing were achieved in India for eight species of damselfishes *viz.* the three spot damsel (*Dascyllus trimaculatus*), striped damsel (*Dascyllus aruanus*), the blue damsel (*Pomacentrus caeruleus*), the peacock damsel *P. pavo,* the bluegreen damsel (*Chromis viridis),* the filamentous tail damsel *Neopomacentrus cyanomos,* the yellowtail damsel (*Neopomacentrus nemurus)* and the Sapphire devil damsel (*Chrysiptera cyanea*). (Gopakumar and Santhosi, 2009; Gopakumar *et al.,* 2009; Gopakumar *et al.,* 2002; Pananghat Vijayagopal *et al.*, 2008).

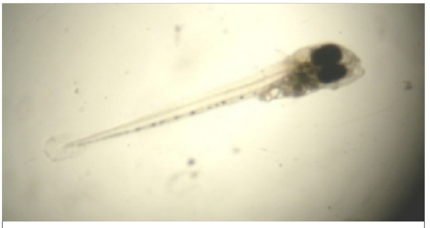
The damsel fishes exhibits protogynous hermaphroditism and they are group/harem spawners. One male can fertilize the eggs deposited by more than one female. The eggs are deposited on any hard substratum as in the case of clown fishes. Earthen pots or PVC pipes can be used as substratum for egg deposition in case of damsel fishes. The egg hatches out on 4th day. The egg size and larval size is comparatively smaller than clown fishes due to which the live feed also have to be smaller to suit the mouth size of the larvae. Hence copepod nauplii/copepodite of suitable size range and nutritional value has to be provided for first feeding of the damsel fish larvae. This is the major difference in seed production between clown fishes and damsel fishes. The mass production of suitable copepod species was a major bottleneck in the seed production of damsel fishes till very recently. However, techniques for culture of copepod species with suitable size and nutritional value have been developed by Vizhinjam Research Centre of ICAR CMFRI, which is being adopted for the seed production of damsel fishes. After the initial feeding stage with copepod nauplii or copepodites, rotifers can also be used as live feed along with green water technique. The rest of the larval rearing techniques are similar to that of clown fishes.

Some commonly bred damsel fishes

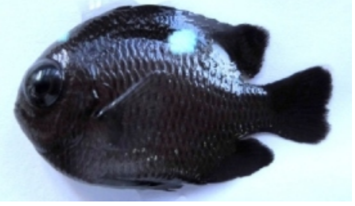
Saphire devil damsel

Fully grown embryo of sapphire devil damsel

Green chromis



Larva of Green chromis



Three spot damsel

Larva of Three spot damsel

Yellow tail damsel

Larva of yellow tail damsel

Humbug damsel

Larvae of Humbug damsel

LIVE FEED CULTURE

Live feed culture is an integral part of marine ornamental fish seed production technique because the initial feeding of the larvae is purely on live feeds such as rotifers or copepods. To culture these zooplankton, micro algae (phytoplankton) is necessary. Hence live feed comprises of micro algae and zooplanktons (such as rotifers, copepods and *Artemia* nauplii).

MICROALGAE

Microalgae constitute the first link in the oceanic food chain. In aquaculture, microalgae are produced as a direct food source for various filter feeding larval stages of organisms. They are also used as an indirect food source in the production of rotifers, *Artemia* and copepods which in turn are used as food for the carnivorous larvae of many of the marine fish species. For rearing marine fish larvae according to the 'green water technique' microalgae are used directly in the larval tanks. This technique is nowadays a normal procedure in marine larviculture and is reported to improve fish larval growth, survival and feed ingestion. The role of microalgae in the rearing water is attributed to (i) providing of nutrients directly to the larvae (ii) contributing to the preservation of live prey nutritional quality (iii) promoting changes in the visual contrast of the medium and its chemical composition and (iv) playing an important role in the microflora diversification of larval gut. Whenever microalgae are used as a direct food source or as an indirect food source in the production of rotifers, *Artemia* or copepods, growth of the animals is usually superior when a mixture of several microalgal species is used.

Recently commercial microalgal products are developed which can also be effectively employed for larviculture. These include microalgae concentrates, frozen and freeze dried microalgae and microalgal pastes. Results of these products are generally good.

Microalgae like *Chlorella sp., Nannochloropsis sp., Tetraselmis sp., Dunaliella sp*., and *Pavolova sp*., *Isochrysis sp.* can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery use.

Nutrition required by Microalgae

- Macro elements include nitrate and phosphate as prominent nutrients
- Micro elements are iron, molybdenum, copper, zinc, cobalt, B1 (thiamine), B12 (cyanocobalamin) and biotin.

Growth phase of Microalgae

Microalgae grow by normal cell division and cultures supplied with excess resources will normally exhibit an exponential increase in cell numbers. After inoculation, the cultures need to acclimate to the new culture condition and the microalgae culture will have a lag or induction phase. When the cells have become acclimatized to the conditions of excess resources, they grow and divide faster and the culture will follow an exponential growth phase, the cell density increases, while the nutrient in the growth medium will be exhausted and the increased density of the algal cells will increase self spreading of the culture. The culture will then come into a phase of declining growth rate before it reaches the stationary phase. In the stationary phase, the net increase in cell numbers is zero. After some time depending on the species, the culture will continue into the death phase, where mortality rate exceeds growth rate.

Micro algae culture methods

The pure strain of micro algae have to be maintained as stock culture and mass culture can be done to meet the requirements for zoo plankton/larviculture. The major methods are indoor culture in which the isolated species are maintained as stock in small container under controlled condition in an aseptic algal culture laboratory, whereas mass scale can be carried out indoor as well as outdoor in which production relies on natural conditions. The rate of growth varies depending upon the type of algae and its culture condition. The general culture methods are stock, sub and mass culture.

(a) Stock culture

The pure stock can be maintained in test tubes. About 20 ml of algal inoculum is required to be cultured every 6 to 7 days in 250 ml flask plugged with cotton-wool containing sterilized seawater with suitable medium: Walne's or Guillard f or G f/2 or Gf/4 or Miquels or TMRL medium in appropriate proportion and needs to be maintained in algal culture laboratory at temperature range of 21-25°C. These are to be frequently sub-cultured to maintain the culture in the exponential growth phase which is the key factor for the successful and efficient algal production system for mass culture to feed the larvae of fishes.

Conway or Walne's Medium

A. Potassium nitrate	-	100 g
Sodium orthophosphate	-	20g
EDTA (Na)	-	45 g
Boric acid	-	33.4 g
Ferric Chloride	-	1.3 g
Manganese chloride	-	0.36 g
Distilled water	-	1 litre
B. Zinc chloride	-	4.2 g
Cobalt chloride	-	4 g
Copper sulphate	-	4 g
Ammonium molybdate	-	1.8 g
Distilled water	-	1 litre

C. Vitamin B1 (Thiamin) - 200 mg in 100 ml distilled water

Vitamin B12 (Cyanocobalamine) - 10 mg in 100 ml distilled water

Prepare A , B and C in different reagent bottles. Add 1ml of A , 0.5ml of B and 0.1ml of C to one litre of filtered and sterilized seawater.

(b) Sub culture

To maintain sufficient algal inoculum for upscaling algal production, the stock cultures must be sub-cultured once in 7 days or depending upon the growth phases of algae. Subculture can be carried out through inoculation of cells from an old stock culture into 3 or 4 l of haufkins flask with fresh culture medium to enable the cells to continue multiplication, growth and needs to be maintained in algal culture laboratory.

(c) Mass culture

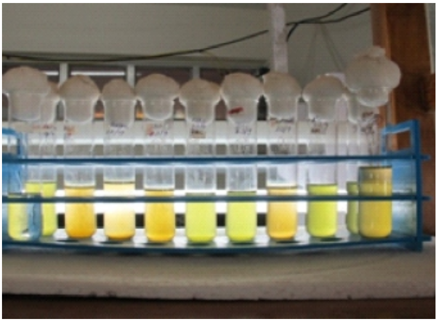
In order to meet the requirements of zooplankton culture, and fish larviculture, the algae need to be produced in large quantity in minimum period of time either indoor or outdoor with suitable culture media. Indoor with transparent roofing is the ideal situation for getting non contaminated algae. Fully-grown culture from the stock culture can be used as inoculum to avoid contamination in mass culture. Mass culture can be carried out 10 l. plastic buckets or 20 l glass carbuoys or 100 and 250 l perspex and glass tanks or 500 l capacity FRP tanks. For large volumes, bigger tanks (such as 2 ton, 5 ton or 10 ton capacity) can also be used for mass culture.

For mass culture, commercial fertilizers can be used instead of the culture media so as to reduce the cost, as detailed below:

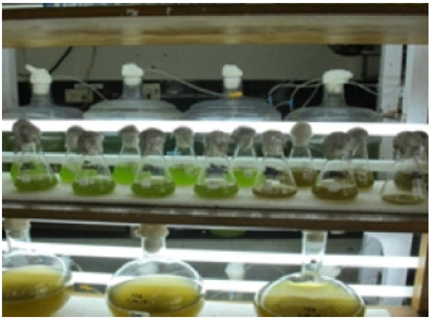
Ammonium sulphate – 100 gm per ton of water

Urea – 10 gm per ton of water

Super phosphate – 10 gm per ton of water



Pure culture in Test tubes



Conical flask culture

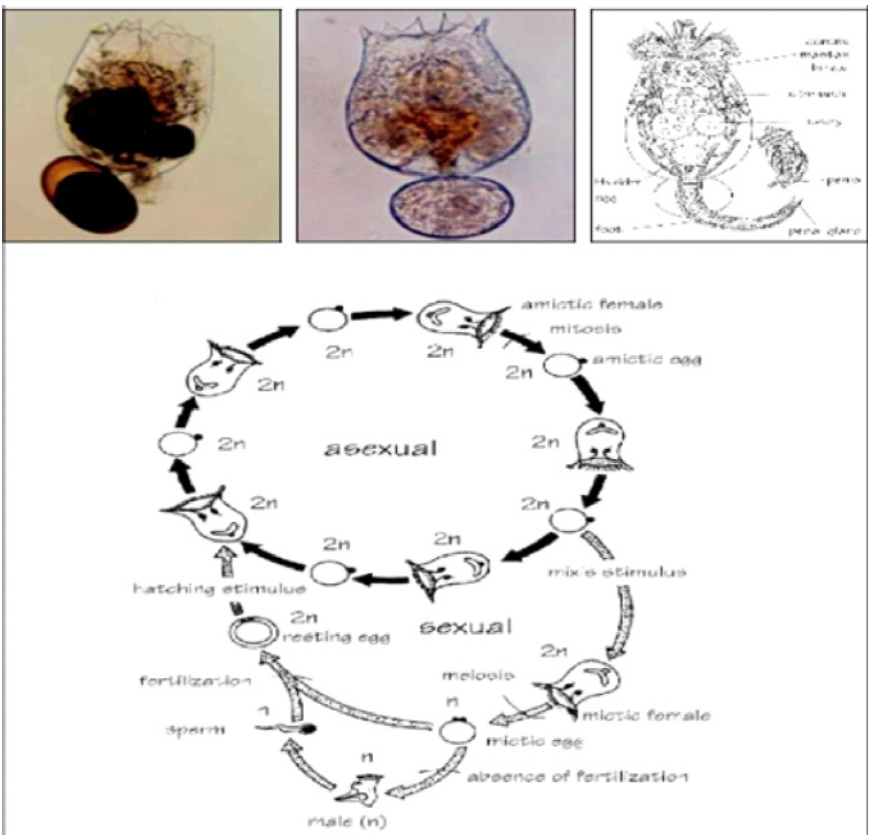
Erlen Meyer flask culture

Culture in Carbuoys

Out door mass culture of micro algae

ROTIFER CULTURE

The rotifers, *Brachionus plicatilis* and *B. rotundiformis* are widely employed for feeding marine fish larvae. Its body size ranging from 70 - 350 microns, (depending on strain) makes these organisms an appropriate prey to start feeding after the resorption of the yolk. Rotifers are used as first food during a few days or weeks depending on the reared species. The main advantages of rotifers include – 1). High growth rate, 2). Filtration of particles in suspension, 3). tolerance to culture conditions and handling, and 4). Appropriate energy content and reasonable nutritional value. In addition, it is relatively modifiable by dietary manipulation by means of post-culture enrichment.



Rotifers are the smaller size zooplanktons widely used in marine fin fish hatchery operations. The marine fin fish larvae initially feeds on the such smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depend on the mouth size of the fish larvae, digestibility and nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are *Brachionus plicatilis* and *Brachionus rotundiformis.*

Pure/stock culture of Rotifer

This can be done in plastic buckets/ troughs. Microalgae such as *Chlorella sp*, *Tetraselmis sp*, *Dunaliella* and *Isocrysis sp* can be used as feed for rotifer.

Mass culture of Rotifer

- Outdoor mass culture can be carried out in volume range of 5 – 12 m^3 (1 tonne, 2 tonne, 5 tonne, 10 tonne)
- Phytoplankton has to be added to rotifer culture tank with density 3-4 million cell/ml for starting the rotifer culture. Rotifers have to be inoculated into tank with initial density 50-100 rotifers/ml
- The next day, algae has to be added into rotifer culture tank
- Rotifers can also be cultured with yeast, however, the nutritional value of such rotifers will be minimal and hence suitable enrichment has to be done before feeding to the fish larvae.

Rotifer Enrichment methods

- Enrichment is done to enhance the nutritional value of rotifer.

- Rotifers in each culture tank can be fed with concentrated microalgae of *Nannochloropsis* ($1x10^4$) every 8 hrs and yeast once in a day at morning.
- Readily available enrichment media can also be used as per the instructions given by the manufacturer of the product.

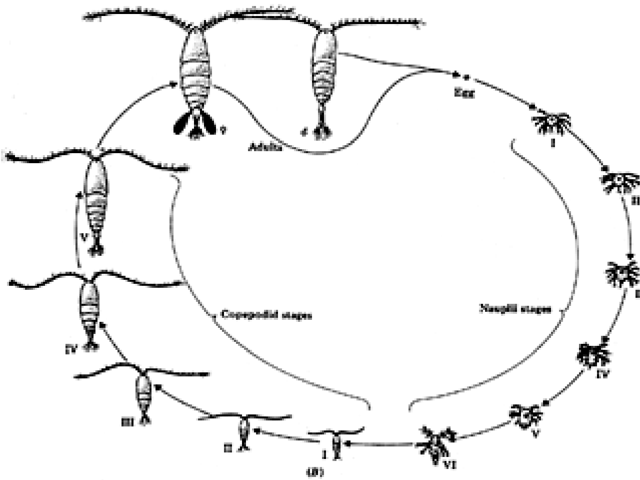
COPEPOD CULTURE

Copepods constitute a first vital link in the marine food chain leading from primary producers to fish. In the open water marine environment, calanoids dominate the herbivorous zooplankton and provide the food chain base for practically all marine fish larvae and planktivorous fish.

The calanoids are predominantly pelagic, occurring at all depths, with some near-bottom and benthic species. They are selective feeders feeding on small phytoplankton cells by filtration or predators feeding on a variety of animal prey including copepod eggs. They are distinguished by their long antennules, as long as the body itself or even longer, with upto twenty seven segments and biramous antennae used as accessory locomotory appendages. The position of the prosome-urosome articulation is between the fifth and sixth post cephalosome somite. The harpacticoids which include over 50% of copepod species are primarily marine, free living, benthic organisms. They inhabit sediments occupying spaces between sand particles (interstitial), burrowing into sediment (burrowers) or living on sediment or plant surfaces (epibenthic). They are distinguished by their short antennules, fewer than ten segments, and biramous antennae. The position of the prosome-urosome articulation is between the fourth and fifth post cephalosome segment.

Some of the major copepod species suitable for larviculture of marine ornamental fishes include *Parvocalanus sp, Oithona sp, Temora turbinata, Pseudodiaptomus sp* etc.

Life cycle of Copepods



Production Methods

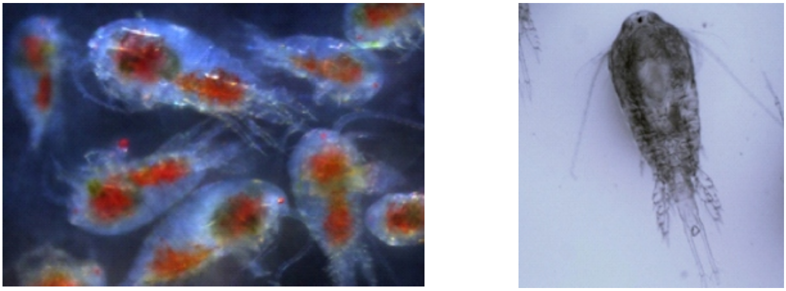
Stock culture

The pure or stock culture of each species should be maintained with aseptic protocols to avoid contamination. This can be carried out in small tanks of 250- 500 L capacity. Feeding with a cocktail of microalgae (such as *Nannochloropsis spp* , *Isochrysis galbana* etc) is ideal. Regular removal of settled matter and topping up with micro algae is essential to maintain the culture in a healthy condition.

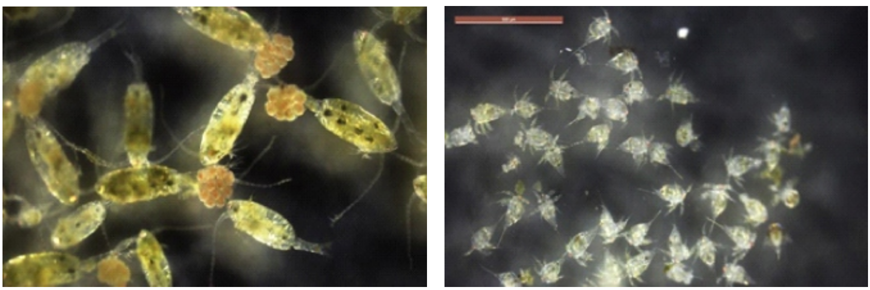
Mass production

Mass production should be done in bigger capacity tanks (FRP or Concrete tanks of 5 ton or 10 ton capacity), since the copepods reproduce by sexual reproduction in contrast to rotifers, which are parthenogenetic. Feeding and tank maintenance can be same as that of stock culture. It is advisable to keep the tanks in areas where natural photoperiod is experienced. Avoiding contamination is the key to success of copepod culture. So the tanks have to be placed in a geographically separated area from other sections.

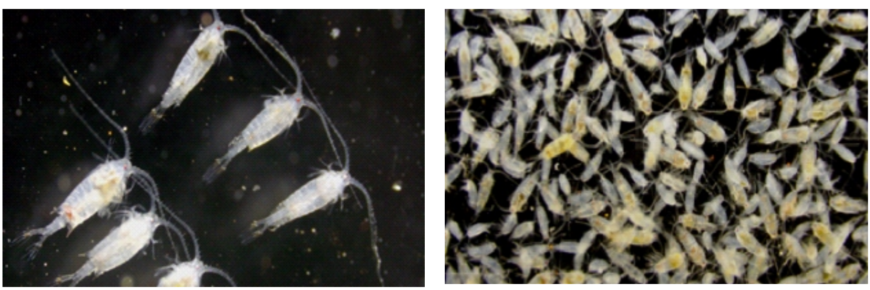
Some common copepods used in fish larviculture



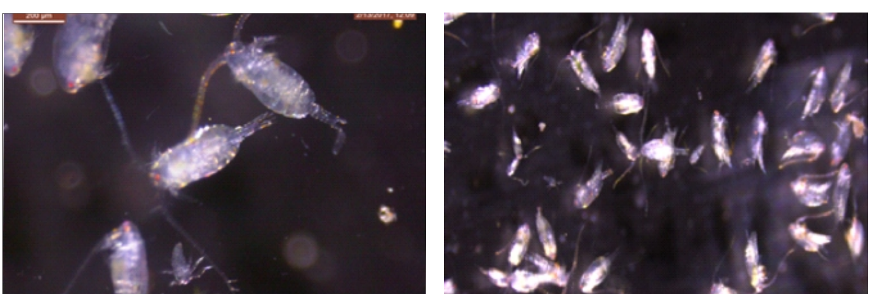
Temora Turbinata



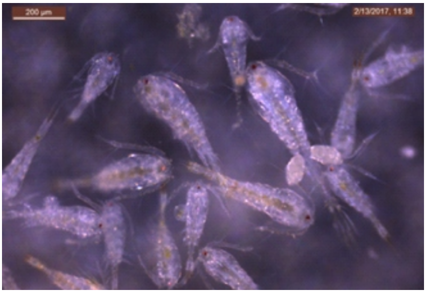
Pseudodiaptomus serricaudatus

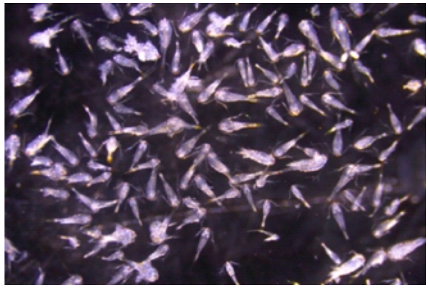


Acartia spinicauda



Parvocalanus arabiensis





Oithona nishidai

ARTEMIA NAUPLII

It is used in marine aquaculture worldwide. Although *Artemia* is not a natural part of marine larvae, it has been favoured due to its convenience for use and high nutritional value. One of its most interesting feature of this organism is its ability to form dormant cysts that are highly resistant to adverse environmental conditions and can be kept viable for years. They are normally stored under dry and cool conditions. The ease and simplicity of hatching brine shrimp nauplii makes them the most convenient and least labour intensive live food available for aquaculture. However, the only negative aspect is its high cost, hence now a days the practical strategy adopted in larviculture of marine species has been to attempt early weaning in conjunction with a prolonged rotifer feeding period to eliminate the use of *Artemia*.

Artemia cyst hatching method

Best hatching results are achieved in containers with a conical bottom, aerated from the bottom with air-lines. Cylindrical or square-bottomed tanks will have dead spots in which *Artemia* cysts and nauplii accumulate and suffer from oxygen depletion. Transparent or translucent containers will facilitate inspection of the hatching suspension, especially when harvesting.

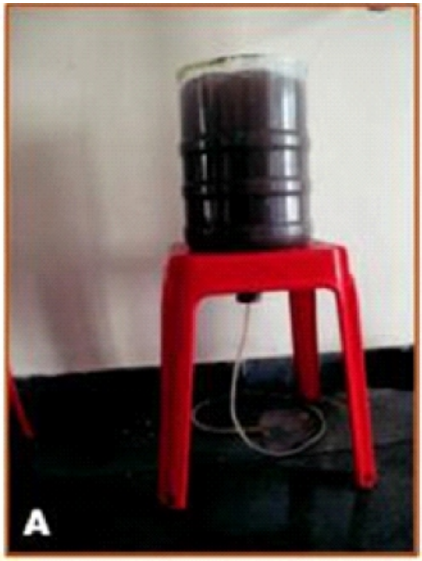
When hatching large quantities of cysts, an impressive bacterial load rapidly develops. Reducing bacterial development during hatching will improve the hygienic status of nauplii and may result in better hatching yields. It can be achieved through simple disinfection of the cysts using liquid bleach solution or through decapsulation.

Disinfection of *Artemia* cysts with liquid bleach

- Prepare 200 ppm hypochlorite solution
- Soak cysts for 30 min. at a density of ± 50 g cysts.l^{-1};
- Wash cysts thoroughly with tap water on a 125 µm screen;
- Cysts are ready for hatching incubation.

***Artemia* hatching**

- Use a transparent or translucent cylindro-conical tank
- supply air through open aeration line down to the tip of the conical part of the tank; oxygen level should be maintained above 2 g.l^{-1}, apply strong aeration
- use filtered natural seawater
- maintain the optimum hatching
- hydrate cysts prior to hatching incubation in tap water for 1-2 hours
- incubate cysts at density of 2 g.l^{-1}; for smaller volumes (<20l) a maximal cyst density of 5 g.l^{-1} can be applied. · Incubate for fixed time period (*e.g*. 20 hr).
- Remove the aeration prior to harvesting
- Wait to separate between nauplii and unhatched cysts. Nauplii will go down to bottom and unhatched cysts will float.
- concentrate nauplii using a light source
- Sieve nauplii, rinse well with tap water.



A. Small scale *Artemia* cyst hatching set up, B. Hatched out *Artemia* nauplii

GROW-OUT METHODS

Indoor grow-out systems

In the case of clown fishes, on 19-20th days of post hatching the larvae metamorphosed into juvenile (size 1.0 to 1.2 cm) and shift from pelagic to epibenthic stages. The rate at which the juvenile fishes grow depends on the size of the tank and stocking density, the quality, quantity of food given and the water temperature. As the clown fishes exhibit social hierarchy, dominant clownfish will grow the fastest and will suppress the growth of other fishes. This can largely overcome by growing the fish up all together in a large tank with sufficient host anemones or dividing the juveniles to several groups in different juvenile rearing tanks of size 250 to 500 lit capacities fitted with biological filters. At this stage, the stocking density need to be reduced to 90-100 numbers of juveniles with single host sea anemone in 100 lit tank capacities for initial one to two months. During the juvenile stages, the fishes show different banding pattern and growth rate and on attaining a size of 24 to 35 mm in total length (TL), the stocking density need to be reduced to 30 to 50 number with single sea anemone in 100 liter tank until marketing. In case of each 500 liter FRP tanks, 130 to 150 juveniles can be reared with 3-4 sea anemones.

In case of Damselfishes, the metamorphosis will be completed around 35^{th} day. A total of about 250 nos. of size 0.8 to 1.2cm can be stocked in the 5 tonne capacity FRP tank for growing up to a marketable size of 2.5 to 3.5 cm for 3 months.

In the grow out phase, a survivability of 70-85% can be obtained through proper feeding with different wet feeds like flesh of sardine, boiled sardine flesh, chopped clam meat, mussel meat and formulated dry feed, for 4 times a day at the rate of 15-20% body weight.

DISEASES AND HEALTH MANAGEMENT

The commonly recognized diseases of aquarium fishes are those that cause visible symptoms externally, whether physical or behavioral. Some of these can be cured or alleviated, while others warn the aquarist to get rid of the fish suffering from them. If the aquarist has no quarantine facilities, it may be necessary to treat the whole tank even it is preferable to medicate the individual fish.

Disease Symptoms and Treatments

Coral fish disease

It is also known as velvet disease, coral disease or salt water itch, and is caused by *Amyloodinium ocellatum*, which is a protozoan parasite. *Amyloodinium* infestations typically begin in the gills. Damage to the delicate gill tissue stimulates fish to produce excessive mucus in the gills, and this condition restricts the exchange of respiratory gases and increases the respiratory pace. As the infestation progresses the cysts become visible on the fin membranes and on the body surfaces. Infected fish often scratch their sides on the bottom or on rocks, and sometimes shake while swimming.

A successful treatment for *Amyloodinium* is treating both the infected fish and the infected tank. If the parasite is not eradicated from the tank, reinfection occurs no matter how effectively the fish have been treated. *Amyloodinium* can be treated successfully with formalin, copper, hydrogen peroxide, malachite green and a number of other compounds. The most common treatment used in large and small marine systems is copper in the form of cupric sulphate complexes with citric acid or chelated with EDTA.

A most effective treatment is freshwater bath. Take freshwater and dechlorinate if necessary. Remove all fish from the infected tank and give them a 1-2 minute bath in the fresh water. The fish can easily withstand the abrupt change in the external osmotic pressure, but the parasites have no protection. They quickly swell and burst. After the freshwater bath the fishes are placed in the treatment tank. The treatment consists of a 3 week exposure to a copper level of 0.2-0.3ppm to destroy all dinospores. Then the fish may be exposed to an antibiotic treatment to control secondary bacterial infections.

Clownfish disease

Brooklynella hostilis is another ciliate which occurs on clownfish and causes 'clownfish disease'. The symptoms appear as small whitish spots with indistinct borders on the sides and sometimes on the fins. These whitish areas begin to enlarge and soon mucus and skin erode off and the affected areas become red. The disease advances rapidly and the fish usually dies within a few days. The formalin treatment recommended for *Cryptocaryon* is suitable for this disease also.

Bacterial disease

A common bacterial disease is the tail rot or fin rot in which red streaks appear on the body or fins which later become ulcers and leads to loss of fins. This is caused by *Pasteurella, Vibrio, Pseudomonas*. Treatment is likely to succeed if trouble is caught very early. Approved antibiotics can used for treatment @ 3-5ppm dose as immersion treatment.

Precautions for effective health management.

- Immediately remove dead and dying fish from aquaria.
- Isolate fish for treatment.
- Identify the disease problem before treatment.
- Change water in the treatment tank every 2 or 3 days.
- Keep the bottom of the treatment tank clean.
- Provide shelter for the fish in treatment.
- Keep light intensity low in the treatment tank.
- Monitor ammonia and nitrite levels in the treatment tank.
- Keep the fish isolated until the cure is complete.
- Monitor copper levels in treatment water every 1 or 2 days.
- Rinse any external filters with fresh water and change the media to prevent reinfection of a tank after the treatment is complete.
- Do not medicate unless necessary.
- Do not continue to add copper without testing the current copper level. The copper level should not exceed 0.3ppm.
- Do not use antibiotic in a tank with a biological filter.

ECONOMICS OF A SMALL SCALE ORNAMENTAL FISH HATCHERY

A typical small-scale hatchery for marine ornamental fish consists of the following units.

1. Broodstock tanks

2. Larviculture tanks
3. Nursery rearing and grow-out tanks
4. One sand filter
5. Outdoor live feed (Phyto and zooplankton) production tanks
6. Seawater and freshwater supply systems.

★ **Advantages of small-scale hatcheries**

1. Low capital inputs
2. Simple construction
3. Ease of operation and management
4. Flexibility
5. Quick economic returns.

✪ **Economic feasibility assessment**

The candidate species selected for economic analysis is the clownfishes

★ **Capital Investment**

This component involves all the expenditure on infrastructure and establishment of the hatchery. Items included in this component generally have a life span larger than one year and they are used to generate the future income from the hatchery.

★ **Operating expenses**

This component is for the expenses that are spent during each production cycle and are essential for the routine operation of the hatchery.

★ **Non-operational expenses**

These are related to the capital cost and investments write off. There are two items under this component for small-scale hatcheries.

i) Depreciation
ii) Interest on capital investment

1. Economics for breeding and seed production of marine ornamental fishes

★ Fixed cost

Capital Investment items	Quantum	Cost in Rs.
Temporary Shed	144m^2 (12 x 12m)	1,10,000
Broodstock (Glass tank – 200 litres)	6 nos	15,000
Larval rearing(Glass tank – 300 litres)	8 nos	36,000
Nursery & grow out (Glass tank–300 litres)	10 nos	45,000
FRP tank (250 liters tank) Larval Stock	5 nos	17,500
FRP tank (1000 liters tank) Larval Stock	3 nos	39,000
Microalgae (outdoor) FRP tank (500 liters tank)	4 nos	26,000
Rotifer (outdoor) FRP tank (500 liters tank)	3 nos	19,500
Sand filter/ Over head tank	1 nos	6,000
Water storage tank (1000 liters)	2 nos	15,000
Artemia hatching tank	2 nos	6,000
4 hp diesel pump	1 nos	19,000
Ozonizer	1 nos	16,000
80w submersible pump	5 nos	15,000
Invertor (1200w)	1 nos	25,000
Air pumps (240 w)	2 nos	15,000
Power installation		10,000
PVC piping, plastic wares (water supply aeration/drainage)	45,000	
Netting, miscellaneous etc.		20,000
	TOTAL COST	**5,00,000**

★ Operating cost

Sl.No.	Items	1st Year (Rs.)	2nd Year(Rs.)	3rd Year (Rs.)
1.	Broodstock fishes/ Anemone	12,000	3,000	3,000
2.	Feeds	6,000	6,000	6,000
3.	*Artemia*	4,000	8,000	8,000
4.	Chemicals for micro algal culture	7,500	7,500	7,500
5.	Electricity	25,000	25,000	25,000
6.	Diesel	25,000	25,000	25,000
7.	Maintenance	12,000	18,000	20,000
8.	Workers' salaries	96,000	1,20,000	1,20,000
9.	Miscellaneous expenditure	12,000	12,000	12,000
10.	Total	1,99,500	2,24,500	2,26,500

Assumptions

There will be six broodstock pairs maintained in the hatchery and at any time there are four active spawning pairs to lay eggs. Each pair will spawn twice a month. An average of 400 larvae is produced during each spawn. The survival rate of the larvae to juvenile phase is 50%. The grow out period from larvae to juvenile is 30 days.

There will be 80% survival rate from juveniles to market size, which is saleable. The period from nursery to market size is 90 days. In a month, 320 saleable size fishes can be produced from one pair of clown fish. Each fish can be sold at a rate of Rs.100.

The first six months of operation will be for construction and setting up of the building, procurement of equipment and collection and maintenance of brooders. The first spawning is expected in seventh month of first year. The sale of the fishes will start from tenth month onwards.

2. Economic performance of marine ornamental fish production

	1st Year (Rs.)	2nd Year(Rs.)	3rd Year (Rs.)
Revenue Sale of clownfish fingerlings @ Rs.100/fingerlings (320 juvenilesx4 pairx2 month= 2,560 numbers 2,560x Rs 100 = Rs. 2,56,000)	2,56,000	-	-
Sale of clownfish fingerlings @ Rs.100/fingerlings (320 juveniles x 4 pair x12 month= 15,360 numbers 15,360 x Rs 100 = Rs. 15,36,000)	-	15,36,000	15,36,000
Subsidy (50% on capital investment)	2,50,000	-	-
TOTAL (Rs.)	5,06,000	15,36,000	15,36,000
Non-operational cost (Rs.)			
a. Depreciation (20%)	1,00,000	50,000	50,000
b. Interest rate on capital @12%	60,000	30,000	30,000
Operating cost (Rs.)	1,99,500	2,24,500	2,26,500
TOTAL EXPENSES (Rs.)	3,59,500	3,04,500	3,06,500
PROFIT (Rs.)	**1,46,500**	**12,31,5001**	**2,29,500**

1. Economics of grow-out unit of marine ornamental fishes

It is also possible to carry out only grow-out of marine ornamental fishes. Here they can buy half-inch size clown fishes and grow them up to 1 ½ inch size in 45 days and market them. The income and expenditure for such activity is mentioned below:

★ **Fixed cost**

Capital Investment items	Quantum	Cost in Rs.
Temporary Shed	$42m^2$ (6 x 7m)	27,000
Nursery and grow out (Glass tank)	6	10,500
Water storage tank (500 liters)	1	07,000
Sand filter/ Over head tank	1	03,000
80w submersible pump	1	15,000
Ozonizer	1	03,000
Air pumps (55 w)	1	03,500
Motor pump	1	05,000
Power installation		10,000
PVC piping, plastic wares (water supply/aeration/drainage)		07,000
Netting, miscellaneous etc.		05,000
TOTAL COST		**96,000**

★ **Operating cost**

Sl.No.	Items	1st Year (Rs.)	2nd Year(Rs.)	3rd Year (Rs.)
1.	Seed	80,000	80,000	80,000
2.	Feeds	3,000	3,000	3,000
3.	Electricity	12,000	12,000	12,000
4.	Maintenance	5,000	5,000	5,000
5.	Miscellaneous expenditure	2,000	2,000	2,000
6.	Total	1,02,000	1,02,000	1,02,000

★ **Economics**

	Amount in Rs. Year 1	Year 2	Year 3
Revenue			
Sale of clownfish fingerlings @ Rs.120/fingerlings (1900 juveniles x 120 =2,28,000)	2,28,000	2,28,000	2,28,000
Non-operating expenses			
a. Depreciation (20%)	9,200	9,200	9,200
b. Interest rate on capital investment @12%	11,520	11,520	11,520
Operating cost	1,02,000	2,28,000	2,28,000
TOTAL EXPENSES	1,32,720	1,32,720	1,32,720
Profit	**95,280**	**95,280**	**95,280**

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